

A21  
cond'it

36. (Amended) A method for increasing the biosynthetic flux in a host cell toward production of an isoprenoid compound, said method comprising, transforming said host cell with a construct comprising as operably linked components, a transcriptional initiation region functional in a host cell, a DNA encoding a tocopherol cyclase, and a transcriptional termination region, wherein said isoprenoid compound is selected from the group of tocopherols and tocotrienols.

A22

40. (Amended) The method according to Claim 39, wherein said plant cell is obtained from a plant selected from the group consisting of *Arabidopsis*, soybean, corn, rice, wheat, leek, canola, cotton, and tomato.

#### REMARKS

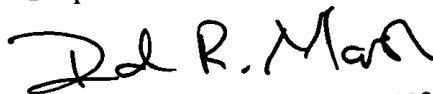
In the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, mailed June 6, 2001, the Office requested submission of a computer readable form of the sequence listing. In conjunction with an accompanying response to the Notice, Applicants submit this Preliminary Amendment.

The specification has been amended to include sequence identification numbers and to correct typographical errors. Support for these amendments can be found throughout the specification (e.g. at page 32, lines 4-24), in the figures, sequence listing, and the original claims. No new matter enters by these amendments. Claims 2-3, 5-9, 11-12, 16, 18, 26, 30-31, 35-36, and 40 have been amended. Support for these amendments can be found throughout the specification (e.g. at page 32, lines 4-24), in the figures, sequence listing, and the original claims. No new matter enters by these amendments. The application presently contains claims 1-41.

The presently pending claims are believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue. The Examiner is respectfully requested to contact Applicant's undersigned representative at 202.942.5071 to address any unresolved issues remaining in this application.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency,  
or credit any overpayment, to our Deposit Account No. 50-1824 referencing matter number  
16515.054.

Respectfully submitted,



David R. Marsh (Reg. No. 41,408)  
June E. Cohan (Reg. No. 43,471)

Date: August 3, 01

ARNOLD & PORTER  
555 12<sup>th</sup> Street, N.W.  
Washington, D.C. 20004  
202.942.5000 telephone  
202.942.5999 facsimile

### Marked-Up Specification

Page 4, lines 4-5:

Figure 1 provides an amino acid sequence alignment between ATPT2 (SEQ ID NO: 2), ATPT3 (SEQ ID NO: 4), ATPT4 (SEQ ID NO: 6), ATPT8 (SEQ ID NO: 12), and ATPT12 (SEQ ID NO: 17) [are] performed using ClustalW.

Page 4, lines 25-26:

Figure 21 provides an amino acid sequence alignment using ClustalW between the *Synechocystis* prenyltransferase sequences, slr1736 (SEQ ID NO: 37), slr0926 (SEQ ID NO: 32), sll1899 (SEQ ID NO: 33), slr0056 (SEQ ID NO: 34), and slr1518 (SEQ ID NO: 35).

Page 4, lines 27-29:

Figure 22 provides an amino acid sequence of the ATPT2 (SEQ ID NO: 2), ATPT3 (SEQ ID NO: 4), ATPT4 (SEQ ID NO: 6), ATPT8 (SEQ ID NO: 12), and ATPT12 (SEQ ID NO: 17) protein sequences from *Arabidopsis* and the slr1736 (SEQ ID NO: 37), slr0926 (SEQ ID NO: 32), sll1899 (SEQ ID NO: 33), slr0056 (SEQ ID NO: 34), and the slr1518 (SEQ ID NO: 35) amino acid sequences from *Synechocystis*.

Page 5, lines 19-20:

Figure 31 is a sequence alignment of the *Arabidopsis* homologue (SEQ ID NO: 113) with the sequence of the public database (SEQ ID NO: 112).

Page 5, line 25-26:

Figure 35 is a sequence alignment of slr1737 (SEQ ID NO: 39), slr1737 *Arabidopsis* homologue (SEQ ID NO: 110) and the *Arabidopsis* chalcone isomerase (SEQ ID NO: 111).

Page 30, lines 6-8:

The sequence encoding ATPT2 prenyltransferase (SEQ ID NO: 1) was cloned in the sense orientation into pCGN8640 to produce the plant transformation construct pCGN10800 (Figure 2). The ATPT2 sequence is under control of the 35S promoter.

Page 30, lines 9-11:

The ATPT2 sequence (SEQ ID NO: 1) was also cloned in the antisense orientation into the construct pCGN8641 to create pCGN10801 (Figure 3). This construct provides for the antisense expression of the ATPT2 sequence from the napin promoter.

Page 30, lines 12-13:

The ATPT2 coding sequence (SEQ ID NO: 1) was also cloned in the sense orientation into the vector pCGN8643 to create the plant transformation construct pCGN10822.

Page 30, lines 14-15:

The ATPT2 coding sequence (SEQ ID NO: 1) was also cloned in the antisense orientation into the vector pCGN8644 to create the plant transformation construct pCGN10803 (Figure 4).

Page 30, line 16 - page 31 line 19:

The ATPT4 coding sequence (SEQ ID NO: 5) was cloned into the vector pCGN864 to create the plant transformation construct pCGN10806 (Figure 5). The ATPT2 coding sequence

(SEQ ID NO: 1) was cloned into the vector TopoTA™ vector from Invitrogen, to create the plant transformation construct pCGN10807 (Figure 6). The ATPT3 (SEQ ID NO: 3) coding sequence was cloned into the TopoTA vector to create the plant transformation construct pCGN10808 (Figure 7). The ATPT3 (SEQ ID NO: 3) coding sequence was cloned in the sense orientation into the vector pCGN8640 to create the plant transformation construct pCGN10809 (Figure 8). The ATPT3 (SEQ ID NO: 3) coding sequence was cloned in the antisense orientation into the vector pCGN8641 to create the plant transformation construct pCGN10810 (Figure 9). The ATPT3 (SEQ ID NO: 3) coding sequence was cloned into the vector pCGN8643 to create the plant transformation construct pCGN10811 (Figure 10). The ATPT3 (SEQ ID NO: 3) coding sequence was cloned into the vector pCGN8644 to create the plant transformation construct pCGN10812 (Figure 11). The ATPT4 (SEQ ID NO: 5) coding sequence was cloned into the vector pCGN8640 to create the plant transformation construct pCGN10813 (Figure 12). The ATPT4 (SEQ ID NO: 5) coding sequence was cloned into the vector pCGN8641 to create the plant transformation construct pCGN10814 (Figure 13). The ATPT4 (SEQ ID NO: 5) coding sequence was cloned into the vector pCGN8643 to create the plant transformation construct pCGN10815 (Figure 14). The ATPT4 (SEQ ID NO: 5) coding sequence was cloned in the antisense orientation into the vector pCGN8644 to create the plant transformation construct pCGN10816 (Figure 15). The ATPT8 (SEQ ID NO: 11) coding sequence was cloned in the sense orientation into the vector pCGN8643 to create the plant transformation construct pCGN10819 (Figure 17). The ATPT12 (SEQ ID NO: 16) coding sequence was cloned into the vector pCGN8640 to create the plant transformation construct pCGN10824 (Figure 18). The ATPT12 (SEQ ID NO: 16) coding sequence was cloned into the vector pCGN8643 to create the plant transformation construct pCGN10825 (Figure 19). The ATPT8 (SEQ ID NO: 11) coding sequence was cloned into the vector pCGN8640 to create the plant transformation construct pCGN10826 (Figure 20).

Additional BLAST searches were performed using the ATPT2 sequence (SEQ ID NO: 1), a sequence in the class of aromatic prenyltransferases. ESTs, and in some cases, full-length coding regions, were identified in proprietary DNA libraries.

Page 32, lines 17-24:

A PSI-Blast profile generated using the *E. coli* ubiA (genbank accession 1790473) sequence was used to analyze the *Synechocystis* genome. This analysis identified 5 open reading frames (ORFs) in the *Synechocystis* genome that were potentially prenyltransferases; slr0926 (annotated as ubiA (4-hydroxybenzoate-octaprenyltransferase, SEQ ID NO:32)), slr1899 (annotated as ctaB (cytochrome c oxidase folding protein, SEQ ID NO:33)), slr0056 (annotated as g4 (chlorophyll synthase 33 kd subunit, SEQ ID NO:34)), slr1518 (annotated as menA (menaquinone biosynthesis protein, SEQ ID NO:35)), and slr1736 (annotated as a hypothetical protein of unknown function (SEQ ID NO:[36] 37)).

Page 36, lines 20-22:

The amino acid sequences for the *Synechocystis* knockouts, slr1736 (SEQ ID NO: 37), slr0926 (SEQ ID NO: 32), slr1899 (SEQ ID NO: 33), slr0056 (SEQ ID NO: 34), and slr1518 (SEQ ID NO: 35), are compared using ClustalW, and are provided in Table 3 below. Provided are the percent identities, percent similarity, and the percent gap. The alignment of the sequences is provided in Figure 21.

Page 37, lines 2-5:

Amino acid sequence comparisons are performed using various *Arabidopsis* prenyltransferase sequences, ATPT2 (SEQ ID NO: 2), ATPT3 (SEQ ID NO: 4), ATPT4 (SEQ ID NO: 6), ATPT8 (SEQ ID NO: 12), and ATPT12 (SEQ ID NO: 17), and the *Synechocystis* sequences, slr1736 (SEQ ID NO: 37), slr0926 (SEQ ID NO: 32), slr1899 (SEQ ID NO: 33), slr0056 (SEQ ID NO: 34), and slr1518 (SEQ ID NO: 35). The comparisons are presented in

Table 4 below. Provided are the percent identities, percent similarity, and the percent gap. The alignment of the sequences is provided in Figure 22.

Page 48, lines 11-15:

The sequences obtained for the homologue from the proprietary database differs from the public database (F4D11.30, BAC AL022537), in having a start site 471 base pairs upstream of the start identified in the public sequence. A comparison of the public (SEQ ID NO: 112) and proprietary (SEQ ID NO: 113) sequences is provided in Figure 31. The correct start correlates within the public database sequence [is] at 12080, while the public sequence start is given as being at 11609.

Page 48, line 24 - page 49, line 3:

The *Arabidopsis* homologue to slr1737 [(SEQ ID xx)] (SEQ ID NO: 110) comprises 488 amino acid residues, has a predicted MW of 55kDa, and [a] has a putative transit peptide sequence comprising the first 98 amino acids. The predicted MW of the mature form of the *Arabidopsis* homologue is 44kDa. The hydropathic plot for the *Arabidopsis* homologue also reveals that it is hydrophilic (Figure 33). Further blast analysis of the *Arabidopsis* homologue reveals limited sequence identity (25[%] sequence identity) with the beta-subunit of respiratory nitrate reductase. Based on the sequence identity to nitrate reductase, it suggests the slr1737 [orf] ORF is an enzyme that likely involves general acid catalysis mechanism.

Page 49, line 10 - line 19:

Multiple sequence alignment was performed between slr1737 (SEQ ID NO: 39), slr1737 *Arabidopsis* homologue (SEQ ID NO: 110) and the *Arabidopsis* chalcone isomerase (SEQ ID NO: 111) (Genbank:P41088) (Figure 35). [65%] Sixty-five percent of the conserved residues among the three enzymes are strictly conserved within the known chalcone isomerases. The crystal structure of alfalfa chalcone isomerase has been solved (Jez, Joseph M., Bowman,

Marianne E., Dixon, Richard A., and Noel, Joseph P. (2000) "Structure and mechanism of the evolutionarily unique plant enzyme chalcone isomerase". *Nature Structural Biology* 7: 786-791.) It has been demonstrated that tyrosine (Y) 106 of the alfalfa chalcone isomerase serves as the general acid during cyclization reaction (Genbank: P28012). The equivalent residue in slr1737 (SEQ ID NO: 39), and the slr1737 *Arabidopsis* homologue (SEQ ID NO: 110) is lysine (K), which is an excellent catalytic residue as general acid.

**Marked-up Claims**

2. (Amended) [An] The isolated nucleic acid sequence according to Claim 1, wherein said tocopherol cyclase is active in the cyclization of 2, 3-dimethyl-5-phytylplastoquinol to tocopherol.

3. (Amended) [An] The isolated nucleic acid sequence according to Claim 1, wherein said tocopherol cyclase is active in the cyclization of 2, 3-dimethyl-5-geranylgeranylplastoquinol to tocotrienol.

5. (Amended) [An] The isolated DNA sequence according to Claim 4, wherein said eukaryotic cell source is selected from the group consisting of mammalian, nematode, fungal, and plant cells.

6. (Amended) The DNA [encoding] sequence of Claim 5<sub>1</sub> wherein said tocopherol cyclase [protein] is from *Arabidopsis*.

7. (Amended) The DNA [encoding] sequence of Claim 6<sub>1</sub> wherein said tocopherol cyclase [protein] is encoded by a nucleotide sequence of SEQ ID NO: 109.

8. (Amended) The DNA [encoding] sequence of Claim 7<sub>1</sub> wherein said tocopherol cyclase [protein] has an amino acid sequence of SEQ ID NO: 110.

9. (Amended) The DNA [encoding] sequence of Claim 4<sub>1</sub> wherein said tocopherol cyclase [protein] is from a source selected from the group consisting of *Arabidopsis*, soybean, corn, rice, wheat, leek<sub>1</sub> canola, [, leek,] cotton, and tomato.

11. (Amended) The DNA [encoding] sequence of Claim 10<sub>1</sub> wherein said tocopherol cyclase [protein] is encoded by a nucleotide sequence of SEQ ID NO: 38.

12. (Amended) The DNA [encoding] sequence of Claim 10, wherein said tocopherol cyclase [protein] has an amino acid sequence of SEQ ID NO: 39.

16. (Amended) A nucleic acid construct according to Claim 15, wherein said nucleic acid sequence encoding tocopherol cyclase is obtained from a source selected from the group consisting of *Arabidopsis*, soybean, corn, rice, wheat, leek, canola, [leek,] cotton, and tomato.

18. (Amended) A plant cell comprising the construct of Claim 13.

26. (Amended) A method for the alteration of the isoprenoid content in a host cell, said method comprising [;] transforming said host cell with a construct comprising as operably linked components, a transcriptional initiation region functional in a host cell, a nucleic acid sequence encoding tocopherol cyclase, and a transcriptional termination region,

wherein said isoprenoid compound is selected from the group consisting of tocopherols and tocotrienols.

30. (Amended) The method according to Claim 29, wherein said plant cell is obtained from a plant selected from the group consisting of *Arabidopsis*, soybean, corn, rice, wheat, leek, canola, [leek,] cotton, and tomato.

31. (Amended) A method for producing an isoprenoid compound of interest in a host cell, said method comprising obtaining a transformed host cell, said host cell having and expressing in its genome:

a construct having a DNA sequence encoding a tocopherol cyclase operably linked to a transcriptional initiation region functional in a host cell,

wherein said isoprenoid compound is selected from the group consisting of tocopherols and tocotrienols.

35. (Amended) The method according to Claim 34, wherein said plant cell is obtained from a plant selected from the group consisting [wherein said compound selected from

the group] of *Arabidopsis*, soybean, corn, rice, wheat, leek    canola, [, leek,] cotton, and tomato.

36. (Amended) A method for increasing the biosynthetic flux in a host cell toward production of an isoprenoid compound, said method comprising,

transforming said host cell with a construct comprising as operably linked components, a transcriptional initiation region functional in a host cell, a DNA encoding a tocopherol cyclase, and a transcriptional termination region,

wherein said isoprenoid compound is selected from the group of tocopherols and tocotrienols [,].

40. (Amended) The method according to Claim 39, wherein said plant cell is obtained from a plant selected from the group consisting of *Arabidopsis*, soybean, corn, rice, wheat, leek    canola, [, leek,] cotton, and tomato.